

CLAIMS

What is claimed is:

1. A method of stimulating bone growth at a site in a subject in need of osteoinduction, said method comprising the step of administering to the site a therapeutically effective amount of an agonist of the non-proteolytically activated thrombin receptor.
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2. The method of Claim 1 wherein the site is in need of a bone graft.
3. The method of Claim 1 wherein the site is a segmental gap in a bone, a bone void or at a non-union fracture.
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4. The method of Claim 1 wherein the agonist is a thrombin peptide derivative comprising a polypeptide represented by the following structural formula or a physiologically functional equivalent thereof:

Asp-Ala-R;

wherein R is a serine esterase conserved sequence.

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- 15 5. The method of Claim 4 wherein the thrombin peptide derivative has between about 12 and about 23 amino acids.
6. The method of Claim 5 wherein the serine esterase conserved sequence has the amino acid sequence of SEQ ID NO. 1 (Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val), or a C-terminal truncated fragment thereof having at least six amino acids, provided that zero, one, two or three amino acids in the serine esterase conserved sequence differ from the corresponding position of SEQ ID NO 1.
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7. The method of Claim 5 wherein the serine esterase conserved sequence has the amino acid sequence of SEQ ID NO. 1 (Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-

Phe-Val), or a C-terminal truncated fragment thereof having at least nine amino acids, provided that zero, one or two of the amino acids in the serine esterase conserved region are conservative substitutions of the corresponding amino acid in SEQ ID NO 1.

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5 8. The method of Claim 5 wherein the serine esterase conserved sequence has the amino acid sequence of SEQ ID NO 2 (Cys-X₁-Gly-Asp-Ser-Gly-Gly-Pro-X₂-Val, wherein X₁ is Glu or Gln and X₂ is Phe, Met, Leu, His or Val), or a C-terminus truncated fragment of SEQ ID NO 2, said fragment having at least six amino acids.

SEARCHED 10 9. The method of Claim 8 wherein the thrombin peptide derivative comprises the amino acid sequence Arg-Gly-Asp-Ala (SEQ ID NO 3).

10. The method of Claim 9 wherein the thrombin peptide derivative comprises the amino acid sequence Arg-Gly-Asp-Ala-Cys-X₁-Gly-Asp-Ser-Gly-Gly-Pro-X₂-Val (SEQ ID NO 4), wherein X₁ is Glu or Gln and X₂ is Phe, Met, Leu, His or Val.

15 11. The method of Claim 10 wherein the thrombin peptide derivative has the amino acid sequence Ala-Gly-Try-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val- (SEQ ID NO 5), or an N-terminal truncated fragment thereof, provided that zero, one, two or three amino acids at positions 1-9 in the thrombin peptide derivative differ from the amino acid at the corresponding position of SEQ ID NO 5.

20 12. The method of Claim 10 wherein the thrombin peptide derivative has the amino acid sequence Ala-Gly-Try-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val- (SEQ ID NO 5), or an N-terminal truncated fragment thereof, provided that zero, one or two amino acids at

positions 1-9 in the thrombin peptide derivative are conservative substitutions of the amino acid at the corresponding position of SEQ ID NO 5.

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13. The method of Claim 5, wherein the subject is administered a therapeutically effective amount of a physiologically equivalent thrombin derivative peptide comprising a C-terminal amide.

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14. The method of Claim 5, wherein the subject is administered a therapeutically effective amount of a physiologically functional equivalent thrombin derivative peptide comprising Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-CONH₂ (SEQ ID NO: 6).

10 15. The method of Claim 5, wherein the subject is administered a therapeutically effective amount of a physiologically functional equivalent thrombin derivative peptide consisting of Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-CONH₂ (SEQ ID NO: 6).

15 16. The method of Claim 11 wherein the thrombin peptide derivative is administered in a pharmaceutical composition additionally comprising an implantable, biocompatible carrier.

17. The method of Claim 13 wherein the implantable, biocompatible carrier is an osteoconductive matrix.

20 18. The method of Claim 11 wherein the carrier comprises a polylactic acid/polyglycolic acid homopolymer or copolymer.

19. The method of Claim 1 wherein the subject is a farm animal, a companion animal or a laboratory animal.

20. A pharmaceutical composition comprising an implantable, biocompatible carrier and an agonist of the non-proteolytically activated thrombin receptor.

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21. The pharmaceutical composition of Claim 20 wherein the carrier is osteoconductive.

22. The pharmaceutical composition of Claim 21 wherein the thrombin receptor agonist is thrombin peptide derivative comprises a polypeptide represented by the following structural formula or a physiologically functional equivalent thereof:

Asp-Ala-R;

wherein R is a serine esterase conserved sequence.

23. The pharmaceutical composition of Claim 22 wherein the carrier is a biodegradable synthetic polymer.

24. The pharmaceutical composition of Claim 23 wherein the biodegradable synthetic polymer is a polylactic acid/polyglycolic acid homopolymer or copolymer.

25. The pharmaceutical composition of Claim 22 wherein the carrier comprises collagen, fibrin, calcium phosphate salts, calcium sulfate, guanidine-extracted allogenic bone or a combination thereof.

26. The pharmaceutical composition of Claim 22 wherein the carrier is injectable.

27. The pharmaceutical composition of Claim 26 wherein the carrier is a poly(propylene fumarate) solution or a calcium phosphate ceramic paste.

28. The pharmaceutical composition of Claim 22 wherein the pharmaceutical composition is administered as microparticles.

29. The pharmaceutical composition of Claim 22 wherein the pharmaceutical composition is pre-shaped before applying to the site in need of osteoinduction.

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50 30. The pharmaceutical composition of Claim 22 wherein the thrombin peptide derivative has between about 12 and about 23 amino acids.

5 31. The pharmaceutical composition of Claim 30 wherein the serine esterase conserved sequence has the amino acid sequence of SEQ ID NO. 1 (Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val), or a C-terminal truncated fragment thereof having at least six amino acids, provided that zero, one, two or three amino acids in the serine esterase conserved sequence differ from the corresponding position of SEQ ID NO 1.

10 32. The pharmaceutical composition of Claim 30 wherein the serine esterase conserved sequence has the amino acid sequence of SEQ ID NO. 1 (Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val), or a C-terminal truncated fragment thereof having at least nine amino acids, provided that zero, one or two of the amino acids in the serine esterase conserved sequence are conservative substitutions of the corresponding amino acid in SEQ ID NO 1.

15 33. The pharmaceutical composition of Claim 30 wherein the serine esterase conserved sequence has the amino acid sequence of SEQ ID NO 2 (Cys-X₁-Gly-Asp-Ser-Gly-Gly-Pro-X₂-Val) , wherein X₁ is Glu or Gln and X₂ is Phe, Met, Leu, His or Val), or a C-terminus truncated fragment of SEQ ID NO 2, said fragment having at least six amino acids.

20 34. The pharmaceutical composition of Claim 30 wherein the thrombin peptide derivative comprises the amino acid sequence Arg-Gly-Asp-Ala (SEQ ID NO 3).

35. The pharmaceutical composition of Claim 34 wherein the thrombin peptide derivative comprises the amino acid sequence Arg-Gly-Asp-Ala-Cys-X₁-Gly-Asp-Ser-Gly-Gly-Pro-X₂-Val (SEQ ID NO 4), wherein X₁ is Glu or Gln and X₂ is Phe, Met, Leu, His or Val.

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36. The pharmaceutical composition of Claim 35 wherein the thrombin peptide derivative has the amino acid sequence Ala-Gly-Try-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val- (SEQ ID NO 5), or an *N*-terminal truncated fragment thereof, provided that zero, one, two or three amino acids at positions 1-9 in the thrombin peptide derivative differ from the amino acid at the corresponding position of SEQ ID NO 5.

37. The pharmaceutical composition of Claim 35 wherein the thrombin peptide derivative has the amino acid sequence Ala-Gly-Try-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val- (SEQ ID NO 5), or an *N*-terminal truncated fragment thereof, provided that zero, one or two amino acids at positions 1-9 in the thrombin peptide derivative are conservative substitutions of the amino acid at the corresponding position of SEQ ID NO 5.

10 38. The pharmaceutical composition of Claim 30 comprising a physiologically equivalent thrombin derivative peptide, wherein the physiologically equivalent thrombin derivative peptide comprises a C-terminal amide.

15 39. The pharmaceutical composition of Claim 30 comprising a physiologically equivalent thrombin derivative peptide, wherein the physiologically functional equivalent thrombin derivative peptide comprises Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-CONH₂ (SEQ ID NO: 6).

20 40. The pharmaceutical composition of Claim 30 comprising a physiologically functional equivalent thrombin derivative peptide, wherein the physiologically functional equivalent thrombin derivative peptide consists of Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-CONH₂ (SEQ ID NO: 6).

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41. A method of stimulating bone growth at a site in a subject in need of osteoinduction, said method comprising the step of administering to the site a therapeutically effective amount of a peptide having the sequence Ala-Gly-Try-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO 5).

5 42. A method of stimulating bone growth at a site in need of a bone graft in a subject, said method comprising the step of administering to the site a therapeutically effective amount of a peptide having the sequence Ala-Gly-Try-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO 5).

10 43. A method of stimulating bone growth in a subject at a segmental bone gap, a bone void or a non-union fracture, said method comprising the step of administering to the bone gap, bone void or nonunion fracture a therapeutically effective amount of a peptide having the sequence Ala-Gly-Try-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val (SEQ ID NO 5).

15 44. A method of stimulating bone growth at a site in a subject in need of osteoinduction, said method comprising the step of administering to the site a therapeutically effective amount of a physiologically functional equivalent thrombin derivative peptide consisting of the sequence of Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-CONH₂ (SEQ ID NO: 6).

20 45. A method of stimulating bone growth at a site in need of a bone graft in a subject, said method comprising the step of administering to the site a therapeutically effective amount of a physiologically functional equivalent thrombin derivative peptide consisting of the sequence of Ala-Gly-Tyr-Lys-Pro-

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Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-
Val-CONH₂ (SEQ ID NO: 6).

46. A method of stimulating bone growth at a segmental bone gap, a bone void or a
non-union fracture, said method comprising the step of administering to the
5 bone gap, bone void or non-union fracture a therapeutically effective amount of
a physiologically functional equivalent thrombin derivative peptide consisting
of the sequence of Ala-Gly-Tyr-Lys-Pro-Asp-Glu-Gly-Lys-Arg-Gly-Asp-Ala-
Cys-Glu-Gly-Asp-Ser-Gly-Gly-Pro-Phe-Val-CONH₂ (SEQ ID NO: 6).

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